

# Functional Morphology of Prey Ingestion by *Placetron wosnessenskii* Schalfeew Zoeae (Crustacea: Anomura: Lithodidae)

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**Abstract.** The relationship between the morphology and functions of the feeding appendages of first-stage zoeae of the lithodid crab *Placetron wosnessenskii* Schalfeew during ingestion is explored in this study. The preoral chambers of these zoeae are bordered on all sides, with the labrum and mandibles forming the anterior borders, the paragnaths and sternal projection together creating the posterior boundaries, and the maxillules forming the sides. The maxillules are the sole pair of appendages responsible for prey manipulation immediately preceding ingestion. Maxillules are capable of remarkable plasticity of movement, enabling them to grasp, control, and redirect violently struggling prey (*Artemia* sp. metanauplii). The asymmetrical mandibles tear and grind the prey, working against each other with rotating motions.

Two separate ratchet-like coordinations of the appendages were seen, each of which enabled the zoea to maintain a firm grasp on the prey while renewing points of leverage for ingestion. The mandibles hold prey in position while the maxillules regrab it to push it farther into the mouth. Similarly, the labrum holds the prey while the mandibles prepare for a new grinding rotation.

Capture and ingestion of an algal cell by a rapid outward flinging and inward clasp of the mouthparts was seen in one videotaped sequence. Gut fluorescence after introduction of various algal species reveals an ability to ingest a range of particle sizes. This plasticity of feeding behaviors allows the zoeae to ingest a range of food items, and thus meet their nutritional needs.

## Introduction

Behavioral observations, especially in conjunction with detailed morphological descriptions, are valuable in relating form and function of the mouthparts of adult crustaceans. Among decapod crustaceans, feeding behavior has been studied by many authors (*e.g.*, Orton, 1927; Roberts, 1968; Greenwood, 1972; Kunze and Anderson, 1979; Manjulatha and Babu, 1991). In adult crabs, the tasks of manipulating, tearing, shredding, and retaining food are divided among their six pairs of feeding appendages. These small, rapidly moving appendages (the mandibles, maxillules, maxillae, and first, second, and third maxillipeds) are obliquely layered, forming the confines and manipulatory structures of the preoral chamber. Gross accounts of feeding behavior in crustaceans commonly include descriptions of the functions of the outer mouthparts, emphasizing the maxillipeds, in manipulation of food items. Detailed accounts of the movements of the inner mouthparts during ingestion are less common, because these appendages are usually blocked from view either by the food being ingested or by adjacent appendages. Schembri (1982) accounted for the motions of the inner mouthparts in his description of feeding in the brachyuran crab *Ebalia tuberosa* (Pennant), and Alexander and Hindley (1985) described the functional morphology of these appendages during food ingestion by the banana prawn *Penaeus merguensis* De Man.

To date, studies of the functional morphology of decapod zoeal mouthparts have focused on descriptions of morphological features, with few or no behavioral observations available. The development of the feeding apparatus in decapods, with special emphasis on the mandibles and gastric mill, has been extensively reviewed by Factor (1989). Lavalli and Factor (1992) used scanning electron microscopy to detail the functional morphology of mouthparts of

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juvenile and larval lobsters. Decapod zoeae have fewer pairs of functional mouthparts than the adults. The zoeal maxillipeds are used for locomotion, and are not used for feeding until the megalopal stage. In these earlier stages, all the actions involved with manipulation and ingestion of food are therefore divided among the maxillules, mandibles, labrum, and, possibly, maxillae. Although detailed analyses of the motions and functions of the inner mouthparts of decapod zoeae during ingestion have not been made, it is reasonable to expect a situation similar to that found in copepods (Paffenhofer and Lewis, 1989), with the functions of zoeal mouthparts being quite different from those observed in adult crabs.

The species used in this study is *Placetron wosnessenskii* Schalfeew, a somewhat dorsoventrally flattened, long-legged lithodid crab found at depths from 0 to 110 m in rocky areas from the Aleutian Islands in Alaska to Puget Sound, Washington (Hart, 1982). This species exhibits a reproductive pattern typical of lithodids, with mating in the spring, brooding for nearly a year, and hatching between March and early May (Nyblade, 1987). There is a short prezoal stage (several minutes in duration; pers. obs.), followed by four planktonic zoeal stages and one megalopal stage (pers. obs.), during which settlement as a megalopa occurs. A morphological description of the first zoeal stage was published by Haynes (1984), and a description of larval and megalopal development and morphology is currently in preparation (Crain and McLaughlin, unpubl. data). *P. wosnessenskii* was chosen for this study because of the large size of the first-stage zoeae (carapace length is about 3 mm) and their willingness to feed under the conditions necessary for videotape recording.

The present paper relates form to function for the appendages involved in the ingestion of large prey items (*Artemia* metanauplii) and unicellular algae by first-stage *P. wosnessenskii* zoeae. Feeding appendages and accompanying setal types of newly hatched zoeae are described and related to their functions during prey ingestion. Descriptions of appendage functions are based on direct observations and on analysis of videotaped feeding activity of untethered first-feeding zoeae.

### Materials and Methods

Morphological descriptions of the mouthparts and behavioral observations were based on zoeae from two separate broods. The first brood was from an ovigerous female *Placetron wosnessenskii* collected on 5 March 1992 and the second from an ovigerous female collected in late October of 1993. Both ovigerous females were collected from depths of about 13 to 15 m at rocky sites near Anacortes, Washington. Each ovigerous female was transported in a bucket of seawater to Shannon Point Marine Center, where it was transferred to a sea table with continuously flowing natural

seawater and held throughout the hatching period. Upon hatching, normal larvae underwent a brief prezoal stage, after which they shed the prezoal cuticles and swam actively in the sea table as first-stage zoeae. Only actively swimming, healthy zoeae were used in the study. A careful comparison showed no morphological differences among the zoeae or between broods.

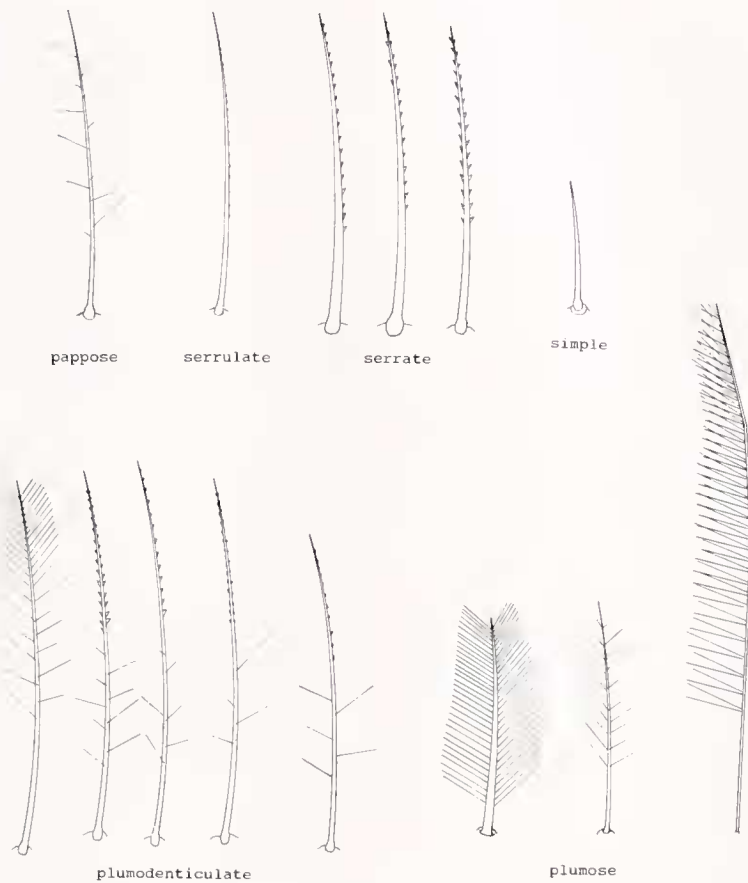
Specimens were preserved in 70% ethanol. Dissection and mounting of preserved specimens in polyvinyl alcohol lactophenol followed staining with 1% chlorazol black (3% in equal parts lactic acid and phenol). Details of appendages and setae were described with the aid of Nomarski differential interference microscopy. Illustrations of morphological features were drawn with camera lucida attachments mounted on dissecting and compound microscopes. Setal descriptions follow the system of Lavalli and Factor (1992) in general terminology and categorization. Terminology used for morphological descriptions follows that of McLaughlin *et al.* (1988).

Appendage movements during ingestion of *Artemia* metanauplii were observed with a dissecting microscope. Because preliminary attempts to tether the zoeae to thin glass rods with cyanoacrylate glue or modeling clay were unsuccessful, newly hatched zoeae (less than 24 h old) were videotaped while they swam freely in a small glass dish containing about 7.5 ml of filtered seawater. During observations of feeding behavior, each zoea was isolated individually in the observation chamber and allowed to adjust to the dish for one to several minutes prior to addition of *Artemia* metanauplii or, in several cases, algal cells. Feeding sequences were videotaped using a black-and-white CCD (charge-coupled device) video camera mounted on a dissecting microscope and connected to a VHS video recorder equipped with an onscreen stopwatch. Frame duration of 1/1000 s enhanced the clarity of the images.

Analysis of videotaped feeding sequences was based on review of the tapes both on normal speed (30 frames per second) and in slow motion using a jog shuttle advance mechanism or stepping through individual frames. Durations of sequences were determined either by use of the onscreen stopwatch or by an actual count of the number of frames used for a given sequence.

Selected frames from ingestion sequences were digitized with Bioscan Optimas image analysis software and illustrated from projected negatives. Illustrations of the ratcheting mechanisms observed from the videotapes were pieced together by copying morphological illustrations into the positions in which the appendages were observed for the relevant sequences.

A preliminary experiment to test the ability of first-stage *P. wosnessenskii* zoeae to capture algal cells was performed using five species of algae. Eight zoeae were isolated in each of six small glass bowls with 75 ml of filtered (2  $\mu$ m) seawater. Each bowl except the control received one of the



**Figure 1.** Setal types found on the mouthparts of first-stage *Placetron wosnessenskii* zoeae. Illustrations not to scale.

following algal species: *Cryptomonas* sp., *Prorocentrum micans*, *Gyrodinium dorsum*, *Chaetoceros calcitrans*, or *Isochrysis galbana*. In bowls containing algae, the concentration of cells was such that the algal cells were visible under a dissecting microscope. The bowls were placed in a dark incubator at 12° C. Zoeae were removed after 12 h, rinsed with deionized water, placed in groups of four on GF/C glass filters, and frozen at 4° C. After 16 h, the filters and zoeae were removed from the freezer, cut into small pieces, and ground in 90% acetone in a tissue grinder. The resultant slurry was poured into volumetric test tubes and refrigerated for 24 h. The tubes were then centrifuged and the supernatant was tested for fluorescence using a Turner fluorometer calibrated with a 90% acetone blank.

## Results

### Types of setae

The types of setae found on the maxillules and maxillae of first-stage *P. wosnessenskii* zoeae (Fig. 1) can be grouped into six of Lavalli and Factor's (1992) categories, differentiated by the armature of the setal shaft. All of these setae

have ampullae and basal septae that are readily visible by differential interference microscopy. Size and shape of the ampulla, prominence of the basal socket, thickness of the walls of the shaft, and shaft length are all variable characters both among and within these six major setal groupings. They are defined as follows:

*Simple setae* (Lavalli and Factor's Type 1). No setules or other secondary processes present on setal shaft.

*Plumose setae* (Lavalli and Factor's Type A). Secondary processes long; thin setules arranged in two distinct rows along length of setal shaft; setule density and distances between rows variable.

*Pappose setae* (Lavalli and Factor's Type B). Secondary processes long; thin setules arranged in no apparent order on setal shaft; setule density highly variable.

*Serrate setae* (Lavalli and Factor's Type D). Secondary processes relatively short; thick, pointed denticles; denticle density variable, size often decreasing along length of setal shaft.

*Serrulate setae* (Lavalli and Factor's Type F). Secondary processes minute scales, often visible only as slight



indentations of outer walls, arranged along setal shaft in one or more rows, concentrated on the distal half.

*Plumodenticulate setae* (Lavalli and Factor's Type C).

Secondary processes not homogeneous; proximal half of setal shaft usually bearing long setules arranged in no apparent order; setules increasing in thickness to form one or two rows of denticles on distal half of shaft, often decreasing in size toward the tip. Secondary process densities variable on all subtypes.

*Morphology and movements of oral region and appendages*

Comparisons of the mouthparts of the zoeae used in behavioral analysis with those used for morphological descriptions showed no differences between the two broods. The morphology and movements of preoral chamber structures and of the appendages expected to be involved in ingestion are as follows:

*Preoral chamber.* The anterior portion of the preoral chamber (Figs. 2, 3) is bordered by the labrum (Fig. 4f), a large, subquadrate lobe with two rows of five or six slender spines medially on the posterior face and a small, dense patch of posteroventrally directed simple setae located between the rows of spines. The observed range of motion of this lobe was limited to a short ventral-to-dorsal movement in which the spines were tilted toward the mouth.

The two ventrally directed lobes of the paragnaths (Figs. 2, 3, 4e) form the posterior lateral borders of the preoral chamber. Each paragnathal lobe is fringed with many fine, anteriorly directed, simple setae along the inner and distal margins. The paragnaths were not seen to move during

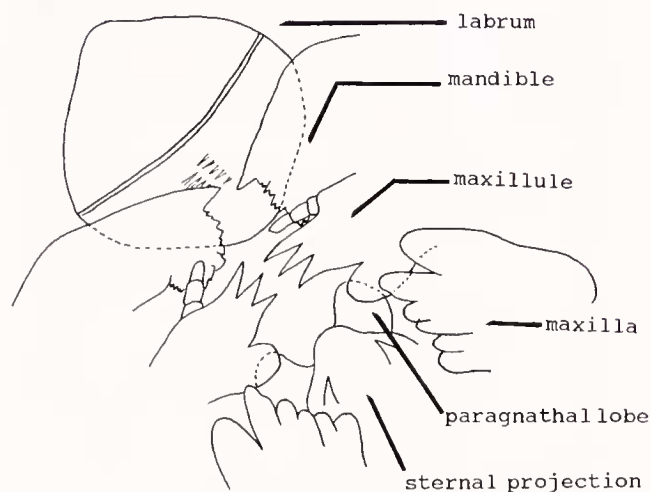


Figure 2. Ventral view of the oral region of a first-stage *Placetron wosnessenskii* zoea, showing locations and orientations of the mouthparts. Setae have been omitted from illustration in order to clearly illustrate appendage positions. Orientation: anterior is top left, posterior is bottom right.

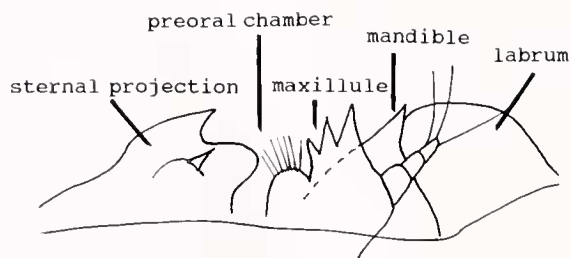


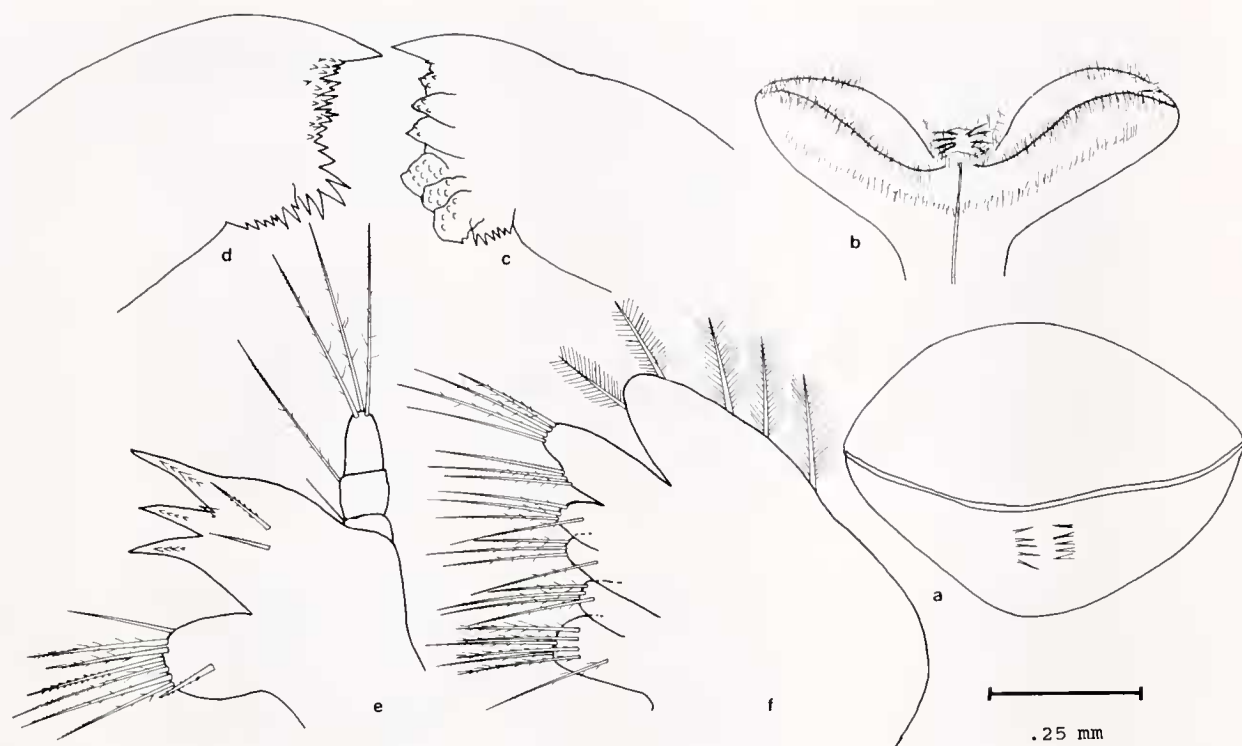
Figure 3. Lateral view of the oral region of first-stage *Placetron wosnessenskii* zoea, showing locations and orientations of the mouthparts. Orientation: anterior is to the right, posterior to the left; ventral is at the top of the figure, and dorsal at the bottom.

ingestion, but were difficult to observe because of their position deep in the preoral chamber.

Posterior to the paragnathal lobes is a large, immobile projection of the sternite (Figs. 2, 3), which completes the posterior boundary of the preoral chamber. The sides of the chamber are bordered, anteriorly to posteriorly, by the highly mobile mandibles, maxillules, and maxillae.

*Mandibles.* The cutting edges of the mandibles are divided into distinct incisor and molar processes and are asymmetrical with respect to the armament of these processes. The incisor process of the left mandible (Fig. 4b) is slightly larger than that of the right (Fig. 4a), consisting of one very large tooth and three or four smaller denticulate teeth. The left molar process is a large, rounded section of the mandible bearing two to four serrate or denticulate ridges culminating in a group of jagged denticles at the extreme (dorsal) edge. The right mandible (Fig. 4a) also bears a large, denticulate incisor process that includes one very large tooth, but in contrast to the left bears approximately 6 to 10 smaller denticulate teeth. The molar region of the right mandible has a complex jagged margin and several serrate ridges. The cutting edges of the mandibles are aligned in a ventrodorsal plane, perpendicular to the sagittal plane of the animal. The incisor processes are positioned farthest ventrally, adjacent to the oral opening.

The mandibles have rolling ventral-dorsal (down-up), mesial-lateral (inside-outside), and anterior-posterior (front-back) components to their movement. Each mandibular motion begins with a lateral-to-mesial movement that brings the cutting edges together and ends with a mesial-to-lateral movement that draws them apart. The ventral-dorsal rolling motions serve to tear and subsequently grind the prey by bringing the two mandibles together in an arcing slice, with contact made first at the extreme (dorsal) ends of the molar regions. Simultaneously, the mandibles make short anterior-posterior motions that grind the ridges of the molar regions against each other. The point of contact between the mandibles is then smoothly shifted from the molars to the incisors over the duration of the arcing movement. Although the actions of the mandibles bring the grinding



**Figure 4.** Illustrations of the preoral chamber features and mouthparts of first-stage *Placetron wosnessenskii* zoeae: (a) labrum; (b) paragnaths; (c) right mandible; (d) left mandible; (e) maxillule; (f) maxilla.

molar regions together first, prey entering the oral region initially contact the tearing incisor processes. As the incisors are drawn across each other, the rolling motion of the mandibles results in a mouthward push of torn material from the incisors to the molar regions, to be crushed by the next mandibular roll. In this way, food is broken into sequentially smaller fragments by the mandibles before entering the oral cavity.

**Maxillules.** Each maxillule (Fig. 4c) has a three-segmented endopodite located distolaterally; a large, toothed, mesially directed basal endite; and a smaller setose coxal endite that is also mesially directed. The setae of the three endopodal segments are (progressing distally): one simple, one pappose, and three plumodenticulate or pappose setae. The basal endite is armed with three large cuspidate teeth, each tooth with two to five denticles, and often with one very small, naked tooth developing between the others. The basal endite also bears two submarginal serrate or serrulate setae. The coxal endite bears six to eight marginal setae that are plumodenticulate, serrate, simple, or a combination of these types and one submarginal serrate seta.

The maxillules position the food prior to ingestion. An overall mesial-lateral motion is most common, with the entire maxillule moving as a lever that uses the basal attachment as the fulcrum, and the tips of the endites describing a large arc. Anterior-posterior motions of the maxillule are also observed, with the base of the appendage again

serving as the fulcrum. The endopodite of each maxillule is muscularized and seems to be capable of some independent motion both in the anterior-posterior and mesial-lateral directions.

The radically different morphologies of the endopodite and coxal and basal endites facilitate the plasticity of functions that this appendage is capable of performing. The denticulate teeth of the basal endite are used primarily for grasping and holding a captured metanauplius. The row of denticles on each tooth presumably aids in maintaining a grip on a struggling metanauplius, and although many of the metanauplii captured struggled energetically, very few of them were able to escape from the maxillules. The two submarginal setae on the basal endite may also aid in this grasping and holding. The three-segmented endopodite was seen to act as an outer guide for captured metanauplii, with the distal setae considerably extending the effective reach of the appendage in the anterior direction. The mobility of this endopodite adds a certain amount of fine control to the positioning of large prey. The location of the coxal endite was often difficult to distinguish in the videotapes, but was clearly at the posterior end of the preoral chamber during the final phase of ingestion. This endite was sometimes used to sweep small fragments of loose food back into the preoral chamber during phases 2 and 3 of ingestion. The setae of the coxal endites of the two maxillules were also seen to overlap each other along the midline of the zoea when the

maxillules were not in motion, thus forming a stationary border to the preoral chamber when at rest.

*Maxillae.* Each maxilla (Fig. 4d) of the first zoeal stage consists of a large scaphognathite (exopodite fused with epipodite) on the lateral portion of the appendage, a weakly bilobed endopodite and two bilobed endites on the mesial portion. The scaphognathite bears four to six plumose setae on the outer margin; the proximal lobe is completely fused with the protopod. There are two or occasionally three small groupings (one to five setae per grouping) of setae on the distal portion of the endopodite, in combinations of serrate, plumodenticulate, simple, and pappose setae. The coxal endite is divided into two distinct lobes, with three plumodenticulate or serrate marginal setae and one plumodenticulate or pappose submarginal seta on the distal lobe. The proximal lobe of the coxal endite bears two plumodenticulate marginal setae, a ridge with four or five plumodenticulate setae, and one plumodenticulate submarginal seta. A similar division into two distinct lobes is characteristic of the basal endite, with the distal lobe bearing three plumodenticulate or pappose marginal setae and one plumodenticulate submarginal seta. There are three or four plumodenticulate or pappose marginal setae and one plumodenticulate or pappose submarginal seta on the proximal lobe of the basal endite.

The maxillae beat continuously during ingestion, with the entire appendages moving water over the thoracic area. The scaphognathites, with their fringe of plumose setae, create a large functional "paddle" that increases the amount of current flow over the developing gill buds at the bases of the maxillipeds and adjacent area. This beating pattern is apparently respiratory, as it is in adults, and is undisturbed by the motions of adjacent appendages. Involvement of maxillae in prey ingestion was not observed.

#### *Behavioral observations: ingestion of Artemia metanauplii*

Not all of the videotaped zoeae were observed to feed. Many individuals failed to capture or attempt to capture metanauplii. Those that successfully captured and ingested *Artemia metanauplii* nearly always exhibited an initial period of several minutes during which no metanauplii were captured. This interval prior to capture and ingestion of the first metanauplius tended to be longer than the pauses between subsequent captures. Initial capture preceded a rapid series of capture and ingestion events, after which the zoea was apparently satiated and ceased feeding. At this point, the appendages even failed to respond to direct contact with the mouthparts by an *Artemia metanauplius*. In two separate sequences, the zoea spent several minutes merely holding, twisting, rotating, and finally rejecting a metanauplius captured during the preceding feeding event.

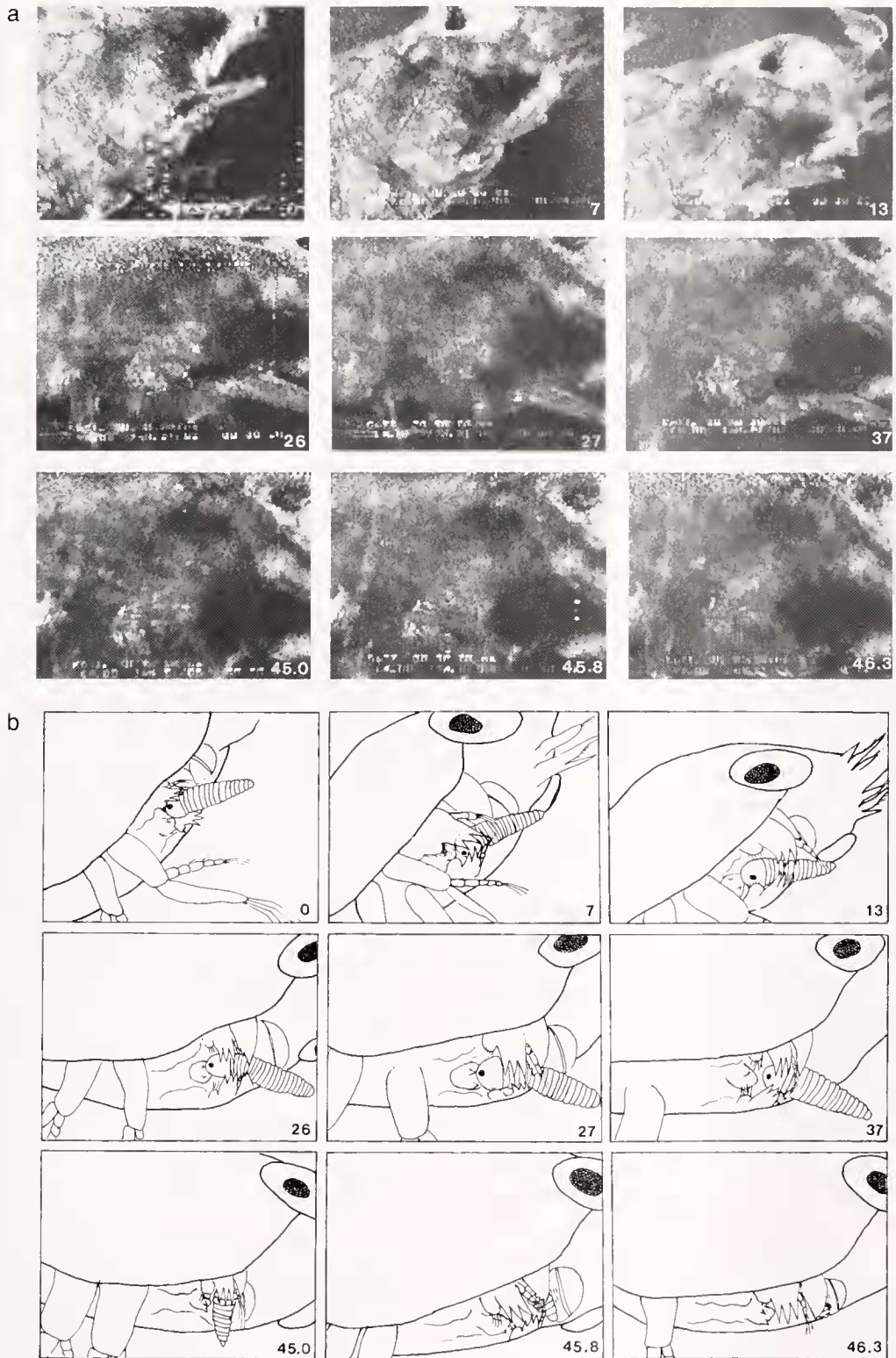
Zoeae captured and ingested metanauplii both while actively swimming and while stationary. Prey manipulation and ingestion were apparently unaffected by movements of the exopodites and endopodites of the maxillipeds, which were used only for locomotion. Similarly, positioning of the abdomen and telson had no apparent effect on ingestion mechanisms.

Because of complications in the use of untethered larvae, compounded by limitations of both field of view and of field of focus, only one sequence was recorded in which the ingestion of an *Artemia metanauplius* was traceable in its entirety (Fig. 5). Partial sequences in which only portions of the ingestion process were recorded were more numerous. Consequently, ingestion events have been separated into three consecutive phases to analyze appendage motions throughout the ingestion process. Each phase is easily distinguished and possesses a distinct beginning and end.

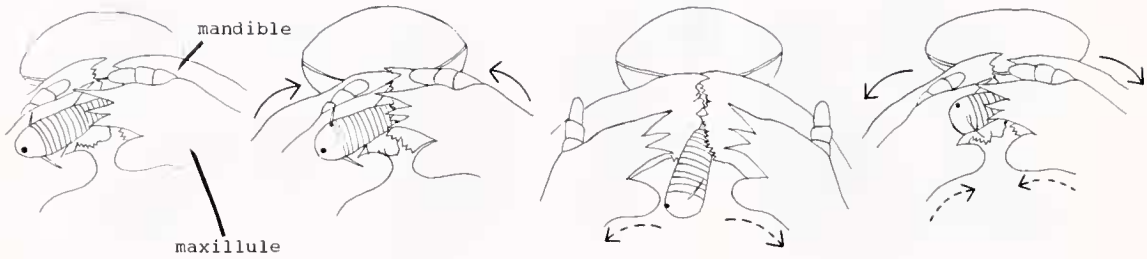
*Phase 1.* During the initial phase of *Artemia* ingestion, only the maxillules are in direct contact with the prey item. As noted above, the principal movements of the maxillules involve the rotating of the entire appendage from the base. In this way, they push the prey around or catch it between opposing endites, and the whole maxillule acts as a large paddle. The maxillules coordinate simultaneous and alternating motions as necessary to manipulate prey into position for ingestion. The first motion of ingestion is a rapid outward fling of the paired appendages, followed by an inward squeezing action, bringing the endites of the maxillules into contact with the metanauplius as it enters the preoral chamber. Metanauplii are held between the basal endites of the opposing maxillules, with the endopodites acting as outer guides and presumably aiding in stabilization of the struggling prey. Symmetrical motions of the opposing maxillules control the anterior-posterior positioning of the metanauplius, while asymmetrical motions are involved with lateral repositioning of the metanauplius or with rotating it about its long axis. In two sequences, the metanauplius was turned end over end by the maxillules employing both symmetrical and asymmetrical motions. Metanauplii can be grasped by the maxillules in any position, but are usually manipulated into either a head-first or tail-first position (with the long axis of the metanauplius perpendicular to the long axis of the zoea) prior to ingestion. In some cases, ingestion sequences are initiated with the metanauplius in a sideways position (with the long axis parallel to the long axis of the zoea), but are completed with the metanauplius in a head-first or tail-first position.

Variations of this basic sequence were seen with two or more metanauplii involved. In four of the five multiple ingestion sequences analyzed, a newly captured metanauplius was used to push a partially ingested metanauplius into the mandibles. In two separate sequences, two metanauplii were ingested simultaneously, although sequential ingestions were far more common. In two of the analyzed





**Figure 5.** Selected frames from a sequence of ingestion of an *Artemia* metanauplius by a first-stage *Placetron wosnessenskii* zoea at 0, 7, 13, 26, 27, 37 (phase 1), 45.0 (phase 2), 45.8 and 46.3 (phase 3) seconds elapsed time: (a) photographs from images captured from a videotaped sequence; (b) illustration drawn from photographs in (a) to enhance clarity.



**Figure 6.** Coordination of maxillule and mandible movements during phase 2 of ingestion of an *Artemia* metanauplius by a first-stage *Placetron wosnessenskii* zoea. Approximate duration: 0.1 to 0.6 s.

sequences, phase 1 of ingestion was shortened to merely the fling and capture steps, which were followed immediately by the maceration associated with phase 2.

Recorded phase 1 duration ranged from 0.2 to 5.9 s in sequences involving only a single metanauplius, and from 6.1 to 233.0 s in sequences involving two or more metanauplii. Mean durations were 3.9 ( $n = 14$ ;  $SD = 5.8$ ) and 56.8 ( $n = 5$ ;  $SD = 93.2$ ) s respectively.

**Phase 2.** This phase of ingestion involves the maxillules, mandibles, and labrum. Phase 2 begins with the first contact of the metanauplius with the mandibles and ends with the loss of direct contact with the prey item by the basal endites of the maxillules. The maxillules, after manipulating the metanauplius into position in phase 1, begin pushing the prey toward the mouth, bringing it into contact with the mandibles. In one sequence, phase 2 was initiated by repeated nudging of the metanauplius into and out of range of the mandibles by the maxillules.

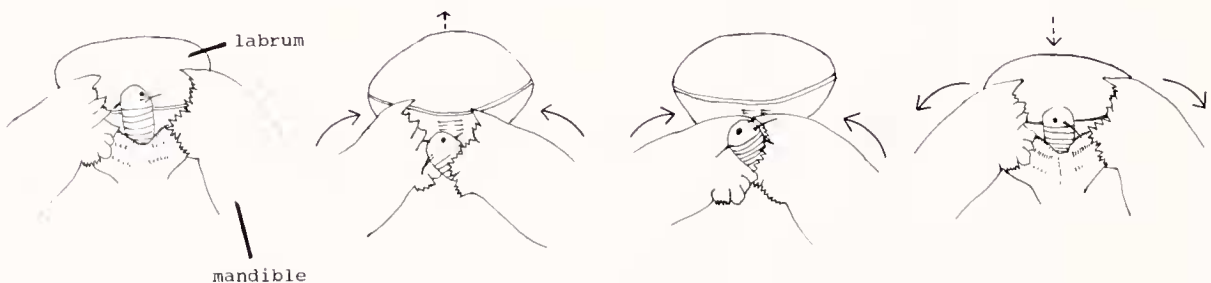
The maxillules and labrum press the metanauplius against the mandibles as the latter tear and grind the prey on its way into the mouth. Two separate ratcheting mechanisms were observed, each of which maintains a steady net movement of the metanauplius into the mouth, while enabling the appendages to renew points of contact for leverage. First, the maxillules and mandibles alternate motions, maintaining a firm grip on the metanauplius as it is masticated (Fig. 6). The maxillules press the prey against the mandibles as they shred bits of tissue with their rolling motions. The mandibles then hold the metanauplius as the maxillules regrasp it

once every two or three mandibular rotations, or as seen in two sequences, with each mandibular roll. Second, the mandibles and the labrum work in conjunction to prevent food from escaping from the mouth when the mandibles are on the recovery stroke of their motion (Fig. 7). The labrum moves downward, pinning the metanauplius in the preoral chamber with its spiny processes as the mandibles return to their initial (lateral) position in preparation for the next grinding roll, with a 1:1 ratio of alternating movements.

The recorded total durations of phase 2 range from 6.5 to 150 s, with a mean duration of 49 s ( $n = 11$ ;  $SD = 45.7$ ).

**Phase 3.** This phase of ingestion begins when the basal endites of the maxillules are no longer in contact with the prey, and continues until the entire metanauplius has passed through the mandibular region into the oral cavity. The mandibles and labrum continue to work together as in phase 2 until the entire metanauplius has been ingested. Although the maxillules are apparently no longer in direct contact with the prey, they often continue to make the sweeping mesial-lateral motions associated with phase 2, and direct small, easily lost fragments of masticated food back into the preoral chamber. A variation of phase 3 was seen in four sequences, when the maxillules pushed one metanauplius through the final stage of ingestion with a second metanauplius.

The range of durations recorded for this phase was from 4 to 84 s, with a mean duration of 23.2 s ( $n = 9$ ;  $SD = 31.6$ ).



**Figure 7.** Coordination of mandible and labrum movements during phases 2 and 3 of ingestion of an *Artemia* metanauplius by a first-stage *Placetron wosnessenskii* zoea. Approximate duration: 0.1 to 0.3 s.



### *Behavioral observations: capture of algal cells*

Although several instances of algal capture by zoeae were observed, only one, in which a *Prorocentrum micans* cell was caught and ingested, was recorded on videotape. This capture took place very quickly, with the maxillules and mandibles drawing laterally, then held open momentarily before closing over the algal cell, which had apparently been drawn into the mouth by the suction created in the oral region. In preliminary gut fluorescence experiments, *P. wosnessenskii* ingested four species of unicellular algae: *Cryptomonas* sp. (approximately  $12 \times 7 \mu\text{m}$ ), *Prorocentrum micans* Ehrenberg (approximately  $39 \times 30 \mu\text{m}$ ), *Gyrodinium* sp. (approximately  $40 \times 35 \mu\text{m}$ ), and *Isochrysis galbana* Parke (approximately  $4 \times 3 \mu\text{m}$ ). These experiments did not contain sufficient numbers of replicates to determine clearance rates, but did indicate that the zoeae were able to ingest a range of particle sizes.

## Discussion

### *Comparison of morphological descriptions*

A comparison of the present description of the mouthparts of first-stage *P. wosnessenskii* zoeae with that of Haynes (1984) reveals few differences. Haynes commented on asymmetry between the left and right mandibles and on the jagged ridge of denticles located at the extreme end of the molar process of each, as noted here. The descriptions of the maxillules and maxillae differed only slightly in terminology and degree of detail.

### *Mechanical functions of setae*

Function can sometimes be inferred by the types and placement of setae on an appendage. Detailed descriptions of setal types and locations, using both light and scanning electron microscopy, have been instrumental in revealing the function of the corresponding appendages or body areas (e.g., Roberts, 1968; Farmer, 1974; Factor, 1978; Ajmal-Khan and Natarajan, 1981; Ohtsuka and Onbe, 1991; Lavalli and Factor, 1992). In some cases specific setal types have been linked to specific functions, especially in feeding and grooming (e.g., Barker and Gibson, 1977; Kunze and Anderson, 1979; Schembri, 1982; Fryer, 1983; Pohle, 1989).

Crustacean setal types have been described in detail by many authors, as reviewed in Jacques (1989). Lavalli and Factor (1992), in their work on the lobster *Homarus americanus*, used light microscopy to produce detailed descriptions of the range and locations of setal types found on the mouthparts of larval and juvenile lobsters. They grouped the setae into 13 categories based on external features, especially the form and position of setules. Each category included variations in form, and several of the setal types

found on the mouthparts of first-stage *P. wosnessenskii* zoeae fit into Lavalli and Factor's (1992) groupings.

The setae found on the tips and edges of the feeding appendages of *P. wosnessenskii* were often more setulose than those on the inner surfaces, a condition that is also reported for crayfish by Thomas (1970), lobsters by Factor (1978) and Lavalli and Factor (1992), and leucosids by Schembri (1982). Farmer (1974) reported smoother setae on the inner surfaces of the mouthparts of *Nephrops norvegicus* (Linnaeus) than on the fringes, attributing this pattern of distribution to the different functions of the two locations. Farmer ascribed primarily a gripping function to the setae located on the insides of the mouthparts, a role for which setulose setae would be less suited because of the recurring need for removal of bits of trapped food. Following this line of reasoning, the submarginal serrulate setae on the inner surface of the maxillules of *P. wosnessenskii* zoeae are well suited for gripping: their minute scales enhance the ability to clutch food without retaining or becoming clogged by small particles. Similarly, the serrate setae on the endites of the maxillules and maxillae may perform gripping functions along with grooming of other setose appendages, as suggested by several authors, e.g., Roberts (1968), Farmer (1974), and Schembri (1982).

A variety of functions have been reported for setae armed with long setules, such as the plumose, pappose, and plumodenticulate setae found on the maxillules and maxillae of *P. wosnessenskii* zoeae. Plumose setae like those found in this study on the scaphognathites of the maxillae aid in generating water currents for respiration (Thomas, 1970; Farmer, 1974; Factor, 1978; Ajmal-Khan and Natarajan, 1981; Schembri, 1982), filter feeding (Rubenstein and Koehl, 1977; Cheer and Koehl, 1987), and locomotion (Fryer, 1983). If the setules are closely spaced, the boundary layer surrounding each will overlap that of adjacent setules, increasing the effective area of the appendage. As the thickness of these boundary layers changes with velocity, a given group of setae can effectively function either as large solid areas or as sieves (Cheer and Koehl, 1987). In this respect, although the planar configuration of the setules on plumose setae may cause them to function as flat paddles, the seemingly haphazard arrangement of the setules on the shafts of pappose setae such as those found on the maxillary endites of *P. wosnessenskii* may in turn cause the setae to function more like cylinders.

Plumodenticulate setae on the maxillules and maxillary endites of *P. wosnessenskii* may act as a combination of pappose and denticulate setae. Schembri (1982) observed that the tendency toward smaller denticles on the distal portions of plumodenticulate setae served to direct stray particles toward the tips of the setal shafts. The large number of these setae on the endites of the maxillae and maxillules of first-stage *P. wosnessenskii* zoeae, coupled with the diversity of secondary processes, suggests that they may

be quite versatile, performing grasping, brushing, cleaning, and current-controlling functions.

The chemosensory and tactile roles of setae have been studied in detail by a number of authors, including Shelton and Laverack (1970), Derby (1982), Atema (1985), and Lavalli and Factor (1992). Regrettably, the electron microscopy and neurological experimentation necessary to attribute chemosensory or tactile function to specific setal types was beyond the scope of this study.

#### *Behavioral and morphological analysis*

The wide ranges of time for each phase of ingestion were due in large part to the variability of levels and types of activity in which the zoeae were engaged. Lapses in ingestion activity were frequent, often corresponding with increased swimming or capture of a second metanauplius, but in some cases not attributable to any obvious external cause. The shortest phase durations were recorded for zoeae that were actively engaged in sequential capture and ingestion events, and were often followed by long periods of reduced interest in feeding.

Although not usually included in most morphological descriptions, the functional significance of the paragnathal lobes warrants mention. It has been observed that in at least some crustaceans the paragnaths are mobile and are mechanically coupled with the mandibles (Wales, 1982). The paragnaths of *P. wosnessenskii* zoeae were not observed to move in this study, which is consistent with the findings of Alexander (1988), who noted that although the paragnaths of anomurans do not move by themselves, they can passively shift position as the mandibles open. Alexander also noted that the many large setae on the inner margins of anomuran paragnaths seem to aid in food retention. This appears to be true in *P. wosnessenskii*.

The labrum is another feature of crustacean anatomy often omitted from morphological descriptions. The functional significance of this structure and its complement of spines and setae in prey ingestion by zoeal *P. wosnessenskii* cannot be overlooked. In all of the observed sequences, the labrum was instrumental in maintaining a steady movement of food into the mouth. Its location at the opening to the oral chamber places it in position to give food items the final push into the foregut as well as to grip large prey items while the mandibles reposition themselves between motions. Similar accounts of labrum and mandible coordination in other crustaceans can be found in Manton (1977) and Schembri (1982).

The mandibles are the masticating appendages, responsible for all tearing, shredding, and grinding of the prey prior to entry into the mouth. Although the rotational motion of the mandibles brings the molar processes into contact with each other before the rest of the cutting edges, the prey item actually contacts the ventrally located incisor processes

first. The incisor portion of the cutting edge tears off large pieces of the prey; the rotational motion of the mandible then aids in pushing those pieces farther toward the mouth to be further shredded and ground by the molar processes. The asymmetry of the molar processes of the left and right mandibles and the complexity of these regions provides a variety of masticating surfaces. As these surfaces are moving past each other, the prey is torn, shredded, and ground into tiny pieces in the final preparation for ingestion.

The maxillules manipulate prey during the initial phase of ingestion. The radically different morphologies of the endopodite and coxal and basal endites facilitate a wide range of functions. The denticulate teeth of the basal endite were used primarily for grasping and holding a captured metanauplius. The row of denticles on each tooth presumably aids in maintaining a firm grip on a struggling metanauplius, and although many of the metanauplii captured struggled energetically, very few of them escaped from the grip of the maxillules. The two submarginal setae on the basal endite may also aid in this grasping and holding process. The three-segmented endopodite acts as an outer guide for the manipulation of captured metanauplii, with the distal setae extending its effective reach in the anterior direction. Endopodite mobility enhances fine control over the positioning of a large prey item. The position of the coxal endite was often difficult to distinguish in the videotapes, but it clearly was at the posterior end of the preoral chamber during the final phase of ingestion. The range of motion demonstrated by manipulation of preserved specimens shows that the coxal endite is capable of at least some movement independent of the rest of the maxillule. Small fragments of floating food were often swept back into the preoral chamber by the coxal endite during phases 2 and 3 of ingestion. The setae of the coxal endites of the two maxillules overlapped each other along the midline of the zoea when the maxillules were not in motion, thus forming a stationary border to the preoral chamber when at rest. Fryer (1983) observed that the naupliar maxillules of the anostracan *Branchinecta ferox* (Milne-Edwards) retained food particles in a similar manner.

The lack of observed feeding function of the endites of the maxillae of *P. wosnessenskii* zoeae is probably related to their more posterior position in the zoeal stages compared to the adults, in which they are presumed to be involved in ingestion. In adult crabs, all of the feeding appendages, including the three pairs of maxillipeds, overlap one another obliquely and are limited in their posterior extension by the sternites of the pereopods. In this arrangement, the maxillary endites are more directly in the path of the food particles than they are in the larvae. Greenwood (1972) observed that the movements of the endites in adult hermit crabs were coupled with those of the scaphognathites, resulting in a continuous beating, "threshing the sides of the food" in the preoral chamber. It is also quite possible that the setae of the



maxillary endites of both zoeae and adult crabs aid in the ingestion of smaller food items either through physical contact with the food or by the creation of a strong feeding current.

*Other possible food sources for zoeae:  
ecological implications*

For some decades, the focus of studies on zoeal diet has been its effect on growth and survival under laboratory and natural conditions (e.g., Kurata, 1959; Sulkin, 1975, 1978; Paul *et al.*, 1979; McConaugha, 1985; Harms and Seeger, 1989; Paul *et al.*, 1989; Epifanio *et al.*, 1991). There is increasing agreement that under natural conditions, zoeae are unlikely to encounter zooplankton prey in the concentrations routinely used in laboratory rearing experiments, and therefore they can probably utilize a variety of food sources (Incze and Paul, 1983; Harms and Seeger, 1989; Paul *et al.*, 1989; Epifanio *et al.*, 1991). Sulkin found that the zoeae of *Callinectes sapidus* Rathbun, a brachyuran crab that cannot successfully complete development to metamorphosis on purely algal diets, nonetheless ingested unicellular organisms (Sulkin, 1975). The lithodid crab *Paralithodes camtschatica* (Tilesius) can be reared in the laboratory on polychaete larvae or *Artemia* nauplii, but not on a diet made up solely of diatoms (Kurata, 1959). However, Paul *et al.* (1989) found that when first-stage *P. camtschatica* zoeae ingested phytoplankton soon after hatching, they molted to the second zoeal stage at higher rates than those that did not. The algal diet did not sustain the zoeae through metamorphosis, and an increasing dependence on carnivory through the zoeal stages was hypothesized for this species (Paul *et al.*, 1989). First-feeding *P. wosnessenskii* zoeae are able to take advantage of a wide range of prey items and probably rely on a variety of planktonic food sources throughout their development.

Factor and Dexter (1993) found that the larvae of the brachyuran crab *Carcinus maenas* (Linnaeus) could capture suspended algal cells, and hypothesized that the setose mouthparts were involved in suspension feeding. In preliminary gut fluorescence experiments, *P. wosnessenskii* ingested unicellular algae covering a range of sizes. How these zoeae capture small algal cells is not known, but we assume that the mechanism is similar to that seen for ingestion of a *Prorocentrum micans* cell. In the one sequence of particle capture recorded on videotape, a *P. micans* cell was captured and ingested using a "fling and clap" method similar to that described for copepods by Koehl and Strickler (1981). The mouthparts were flung outward, enlarging the space between them, thus drawing the cell into the mouth. The mouthparts were then closed over the cell, squeezing water out through the spaces between the setae and endites, as seen in algal capture by copepods (Koehl and Strickler, 1981).

This study demonstrates that *P. wosnessenskii* zoeae can utilize prey items ranging from unicellular algae (*Cryptomonas* sp., *Prorocentrum micans*, *Gyrodinium* sp., and *Isochrysis galbana*) to relatively large, active zooplankton (*Artemia* sp. metanauplii). McConaugha (1985) identified three criteria for suitability of prey items as food sources for larval crustaceans: (1) appropriate size for capture and consumption, (2) adequate concentration, and (3) essential dietary nutrients to meet the larvae's needs for survival, growth, and metamorphosis. Natural plankton assemblages are varied in composition both spatially and temporally. The ability to capture and ingest a variety of sizes and shapes, expanding the diversity of prey species that meet McConaugha's first criterion, increases the probability that the zoea will be able to fulfill its nutritional requirements for successful development.

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